Some virus diseases of grape vine in Czechoslovakia, their purification and diagnosis

J. POZDĚNA and G. VANEK

Czechoslovak Academy of Sciences, Institute of Experimental Botany, Prague, Czechoslovakia
Grapevine and Viticultural Research Institute, Bratislava, Czechoslovakia

INTRODUCTION

Following virus diseases of grape vine [16, 17] occur in Czechoslovakia.

The Bratislava mosaic of grape vine. This disease was described by Blattný and Joska [2]. Early in the spring the leaves usually turn from yellow to yellowish-white in the form of irregular yellow areas. These symptoms disappear by the end of July; in the leaves having strongly developed symptoms the decoloration was visible till the autumn. The growth of the stock is normal. This disease occurs in vineyards rather rarely.

The Mělník mosaic of grape vine [13]. Immediately after budding the leaves turn yellow to yellowish-white, usually along the veins, in patches or on the whole leaf blade. In June the symptoms disappear. When the plants are strongly infected the sprouts shorten, the yeald decreases and the stocks die out. In vineyards the Mělník mosaic is spread in circles.

Necrosis of grape vine [5]. The symptoms appear mostly in July and in August. They manifest themselves by an asymetry of the leaves and by appearence of yellow-green spots. The tissue under the spots becomes yellow and falls.

Fan leaf of grape vine [6]. Infected stocks have very rich foliage. The leaves have desoriented veins, irregular margines and often are wavy. In schoots shortening of internodia and other deformities occur.

Leaf roll of grape vine [12]. The leaves of the diseased plant are convex, rolled and prematurely decolorized. In white varieties white colour takes place; red varieties become red.

Yellow vein of grape vine [4]. The symptoms mostly developed in May and June. The veins and narrow stripes along them are yellow. Grapes are underdeveloped.

Vein banding of grape vine [3]. The symptoms appear in the second half of the vegetation period as light green or yellow margines along the principal veins.

MATERIAL AND METHODS

Experiments were performed with the following four viruses: Mělník mosaic, Bratislava mosaic, Fan leaf and Vein banding.

After transmission on to several host plants *Chenopodium quinoa* was found to be the most suitable host. *Chenopodium murale* and *Chenopodium amaranticolor*, in spite showing sometimes the symptoms of this diseases, are not as reliable as *Chenopodium quinoa*. The same occurs in case of *Petunia hybrida* and the bean Prince.

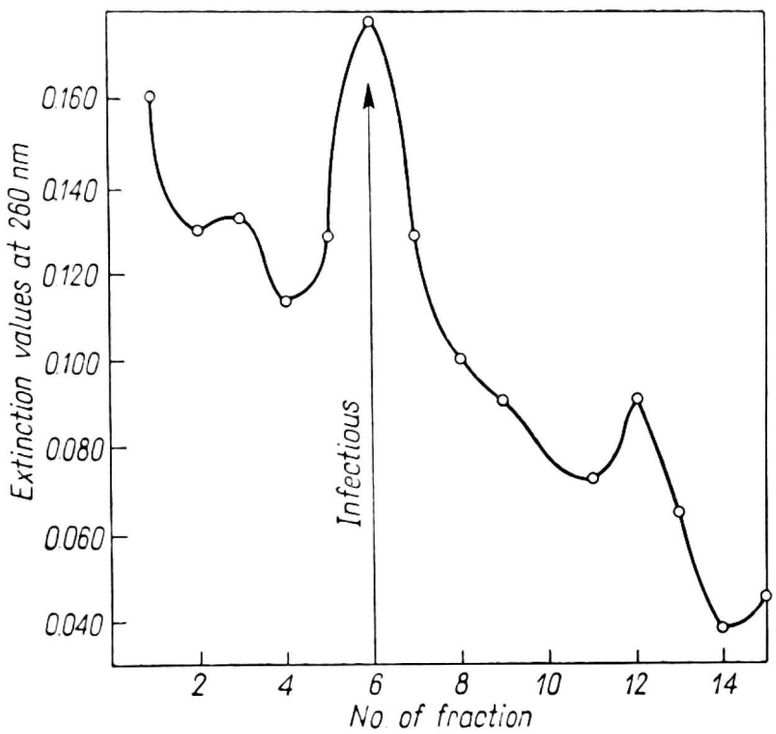

Fig. 1. The curve of protein distribution after the gradient ultracentrifugation of the sap of *Chenopodium quinoa* infected with Bratislava mosaic. The fraction No. 6 is infectious.

On *Chenopodium quinoa* the above mentioned viruses showed following symptoms:

(1) Mělník mosaic — irregular systemic spots on non-inoculated leaves; slight deformation of the leaves.

(2) Bratislava mosaic — decoloration of the veins which starts at the base of the leaf. This symptom occurring even on noninoculated leaves.

(3) Fan leaf — similar symptoms as in case of the Bratislava mosaic.

(4) Vein banding — similar symptoms as in case of the Mělník mosaic. Occurrence of a slight deformation of the leaves.

After transmission onto *Chenopodium quinoa* it was shown that the Mělník mosaic and the vein banding both show similar symptoms, i. e. spots on non-inoculated leaves. Symptoms of the two remaining diseases, i. e. of the Bratislava mosaic and of the fan leaf, manifesting themselves as decoloration of veins on *Chenopodium quinoa*, are similar to each other.

Purification of the viruses mentioned above was performed by a partly modified method according to Taylor and Hewitt [14].

Leaves from the infected plants of *Chenopodium quinoa* were frozen in liquid

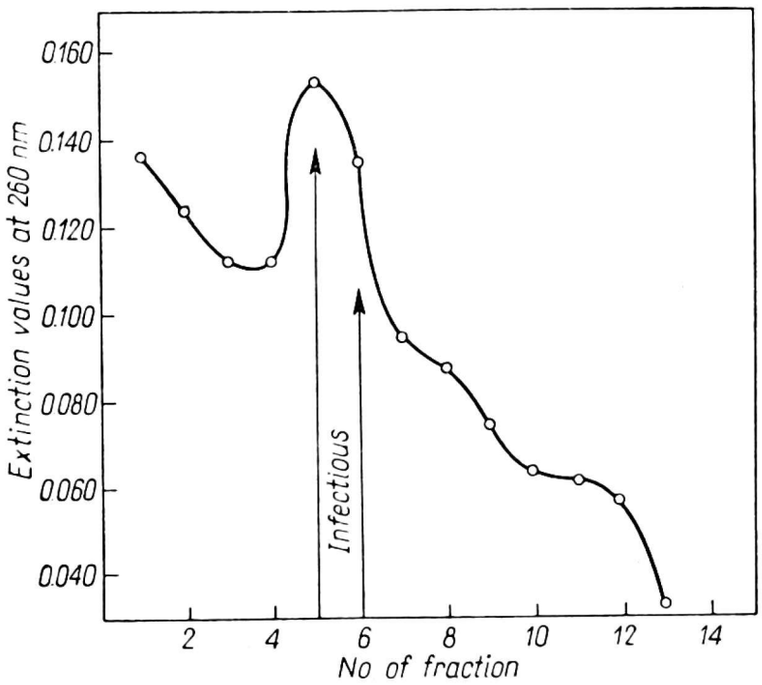

Fig. 2. The curve of protein distribution after the gradient ultracentrifugation of the sap of *Chenopodium quinoa* infected with Fan leaf. The fractions Nos 5 and 6 are infectious.

nitrogen and then rubbed to powder. The material was homogenized in an extract solution, the composition of which was 0.1 M of sodium citrate containing 2% of sodium pyrosulphite. The pH was adjusted to 7.2. For 1 g of the material 2 ml of the extract solution were used. The homogenate was then mixed with the mixture of chloroform-butanol in temperature —16°C in a ratio of 1 ml: 1 g of starting green material. After centrifugation (4,800 rpm during 30 min) the aqueous phase was centrifugated in the bowl 30 of the ultracentrifuge Spinco L during 90 min at 30,000 rpm. The sediments were resuspended in a smal quantity of 0.02 M phosphate buffer pH 7.6 and subdued to a triplex differential centrifugation at

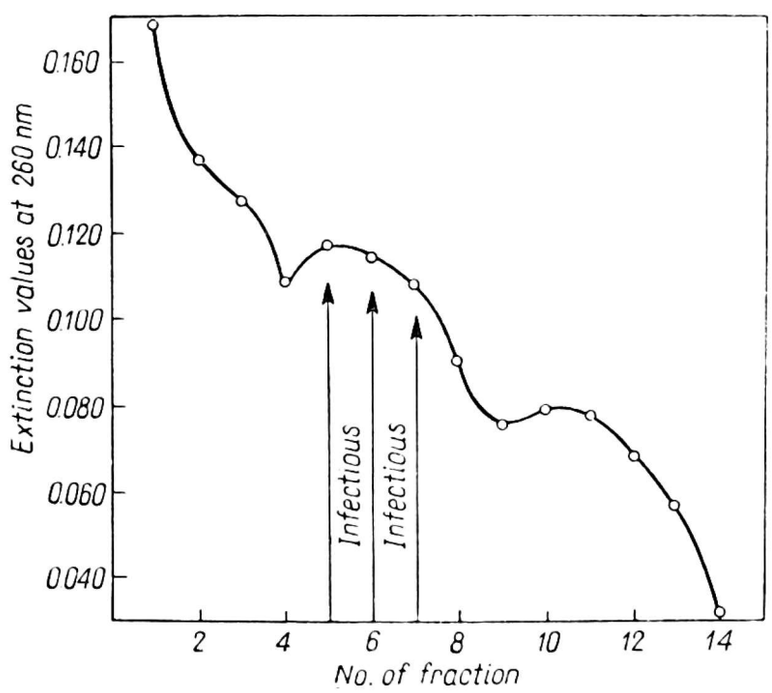

Fig. 3. The curve of protein distribution after the gradient ultracentrifugation of the sap of *Chenopodium quinoa* infected with Mělník mosaic. The fractions Nos 5, 6 and 7 are infectious.

40,000 rpm during 45 min and at 8,500 rpm during 10 min. The final product was superposed on saccharose gradietns, composed of 10, 20, 30 and 40% saccharose solution and centrifugated in SW bowl, during 150 min at 24,000 rpm. After centrifugation cells were perforates and single fractions of 2 ml were taken. Each fraction was then measured on the spectrophotometer VSU2 at a wave-lenght of 260 nm. Measuring of single viruses fractions is represented in graphs. It was shown that Mělník mosaic and vein banding have approximately the same curves. The curves of fan leaf and Bratislava mosaic were also similar, but they differed from those of Mělník mosaic and vein banding. After dialysis single fractions were diluted 1:5 and inoculated on *Chenopodium quinoa.* At the Bratislava mosaic the infectious fraction was the fraction No. 6, at the Mělník mosaic the infectious fractions had Nos 5, 6, 7, infectious fractions of fan leaf had Nos 5 and 6 a those of vein banding Nos 3 and 4. With some of the obtained fractions rabbits were inoculated in order to obtain diagnostic antisera. These experiments have not been finished yet.

Last year serological studies of the Bratislava mosaic [8] were made. The virus was isolated likewise by means of gradient ultracentrifugation. The virus was purified by the method used by Allen [1], Regenmortel and Engelbrecht [9-11], Pozděna, Filigarová and Blattný Jr [7] in case of fruit trees. The method was modified for the needs of the work with grape-vine viruses. The sap was purified by centrifugation at 3,000 rpm during 20 min, then cold chloroform was added. After a double

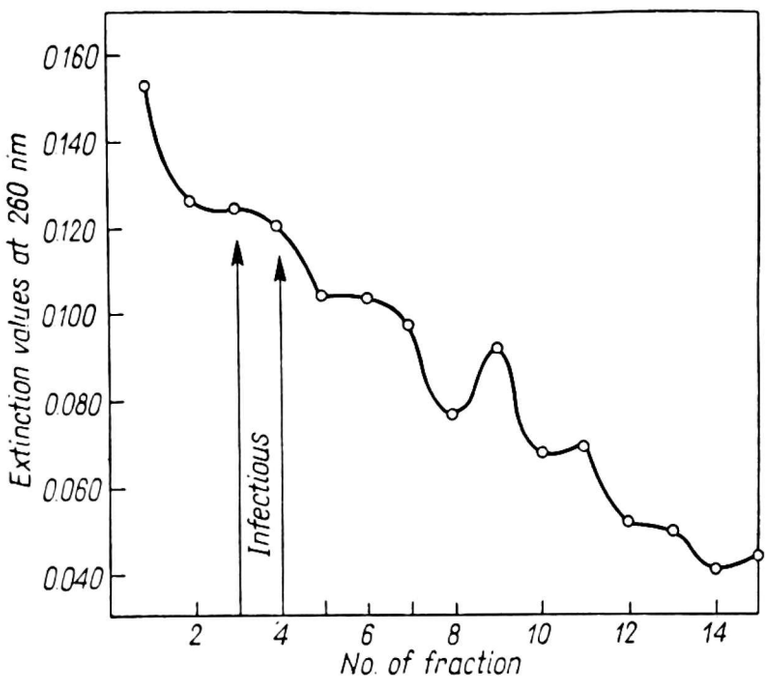

Fig. 4. The curve of protein distribution after the gradient ultracentrifugation of the sap of *Chenopodium quinoa* infected with Vein banding. The fractions Nos 3 and 4 are infectious.

low centrifugation the supernatant was centrifugated at 30,000 rpm during 2 hrs. The sediment was resuspended in a small quantity of water, centrifugated during 15 min at 5,000 rpm and the supernatant superposed on saccharose gradients. After ultracentrifugation the content of the cells was analysed by an automatic registration photometer at a wave-length of 260 nm. Rabbits were immunized with single

fractions. It was shown that the antiserum prepared from the infectious fraction reacted specifically with the Bratislava mosaic virus.

Comparing the curves of the Bratislava mosaic, recorded last year in our experiments with those recorded this year we can observe that the infectious fractions is situated in the same place.

SUMMARY

The diagnosis of the grape vine virus diseases, occuring in Czechoslovakia, can be determined by means of mechanical transmission on *Chenopodium quinoa*. It is not possible to distinguish on the basis of the symptoms on the host plant, the Bratislava mosaic and the fan leaf, as well as the Mělník mosaic and the vein banding. These findings comport with the curves recorded after purification of the viruses by gradient ultracentrifugation. After the gradient ultracentrifugation single fractions were tested on their infectivity and so, the basis for preparation of the antisera was created. Antisera against the Bratislava mosaic were prepared by immunization of rabbits. Infectious fraction was used as antigen. The antisera reacted specifically with the Bratislava mosaic virus.

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